

Exhibit

# MOLECULAR BIOLOGY OF THE CELL

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"Long ago it became evident that the key to every biological problem must finally be sought in the cell, for every living organism is, or at sometime has been, a cell."

Edmund B. Wilson  
*The Cell in Development and Heredity*  
3rd edition, 1925, Macmillan, Inc.

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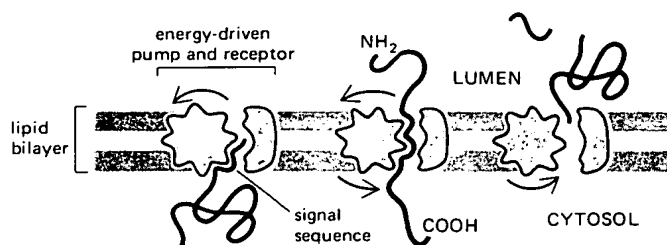
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**Figure 7-29** Schematic view of an energy-driven protein pump. After a receptor recognizes some special feature of an amino-terminal peptide, the pump is activated to force the entire protein through the membrane. A mechanism of this type is apparently needed to account for the observed transfer of already synthesized proteins from the cytosol into mitochondria and chloroplasts.

shown to be required to provide the "signal" that is recognized to direct the protein to the correct site. The membranes of both of these cellular organelles must contain a specific receptor for the appropriate amino-terminal signal. These receptors would seem to be functionally linked to an energy-driven pump that forces the recognized protein across the membrane, possibly unfolding it transiently in the process (Figure 7-29).

Some membrane proteins may enter and partially cross the ER membrane by a posttranslational import mechanism. This is probably the case for proteins that have a large amount of their mass in the lipid bilayer and only small portions exposed on the noncytoplasmic side of the membrane. It is easy to imagine how such a hydrophobic membrane protein could simply dissolve in the lipid bilayer after being released into the cytosol or even during its synthesis. The energy required to transfer a relatively small hydrophilic portion of the protein across the lipid bilayer could come from the gain in free energy associated with the transfer of the hydrophobic portion from an aqueous environment to the hydrophobic interior of the bilayer. Some of the proteins located in the outer membrane of mitochondria appear to enter their membrane in this way following their synthesis on ribosomes in the cytosol (see p. 539).

Even if we consider only the few examples just discussed, it is obvious that many different types of signal sequences must exist on proteins. Some signals will direct a protein to the ER, others to a mitochondrion, and yet others to a chloroplast (in a plant cell). The recognition systems involved must have a high degree of selectivity, since proteins seem to be directed quite reliably to their correct intracellular address.

## Most Proteins Synthesized in the Rough ER Are Glycosylated<sup>22</sup>

**Glycosylation** is one of the major biosynthetic functions of the ER. It is a striking fact that most proteins sequestered in the lumen of the ER before being secreted from the cell or transported to other intracellular destinations (such as the Golgi apparatus, lysosomes, or plasma membrane) are **glycoproteins** (Figure 7-30). In contrast, the soluble proteins of the cytosol are not glycosylated.

An important advance in understanding the process of glycosylation was the discovery that mainly one species of oligosaccharide (composed of *N*-acetylglucosamine, mannose, and glucose) is transferred to proteins in the ER, and that this oligosaccharide is always linked to the  $\text{NH}_2$  group on the side chain of an asparagine residue of the protein (Figure 7-31). All of the diversity of the asparagine-linked oligosaccharide structures on mature glycoproteins results from extensive modifications of this single precursor structure, most of which occur during subsequent transit through the Golgi apparatus (p. 357). The asparagine-linked oligosaccharides (*N*-linked oligosaccharides) are by far the most common ones found in glycoproteins. Often, but less frequently, oligosaccharides are linked to the OH group on the side

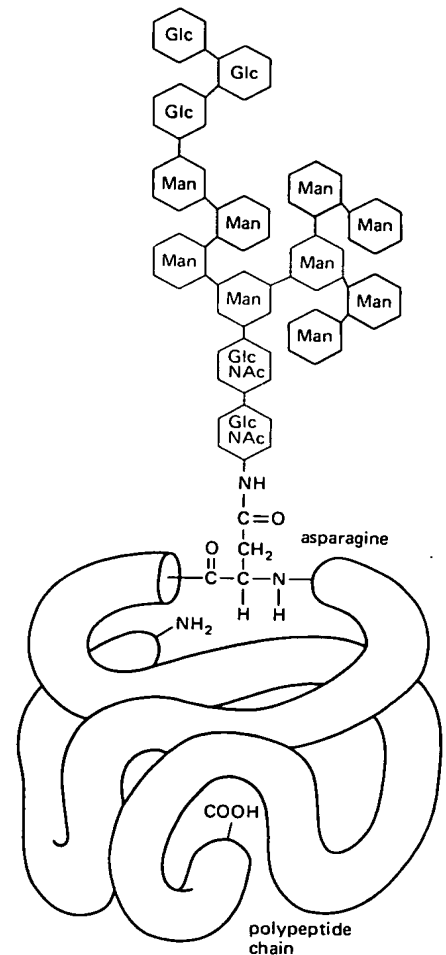
chain of aserine, threonine, or hydroxylsine residue (**O-linked oligosaccharides**); it is not yet clear whether this glycosylation begins in the ER or occurs only in the Golgi apparatus, and the details are as yet poorly understood.

Intracellular sites of glycosylation can be identified by autoradiography. For example, when slices of thyroid are briefly incubated with [ $^3\text{H}$ ]mannose, most of the  $^3\text{H}$  is incorporated into an oligosaccharide attached to thyroglobulin, a major glycoprotein synthesized by thyroid cells. To determine where in the cell this reaction takes place, cells are labeled briefly with [ $^3\text{H}$ ]mannose and then processed for electron microscopy by the method illustrated in Figure 7-32. Thin sections of these cells are coated with a thin layer of photographic emulsion so that sites of  $^3\text{H}$ -disintegration in the section are recorded as silver grains in the overlying film (Figure 7-33). When the section is examined in the electron microscope, the location of  $^3\text{H}$  relative to known cellular structures can be determined. The result of this type of experiment is clear-cut: [ $^3\text{H}$ ]mannose is incorporated only in the ER (Figure 7-34).

### The Oligosaccharide Is Added to the Growing Polypeptide Chain on the Luminal Side of the ER

The actual transfer of the oligosaccharide to the asparagine is believed to take place on the luminal side of the ER membrane, and the enzyme catalyzing this event is a membrane-bound protein with its active site exposed on the luminal surface. This fact explains why cytosolic proteins, which never encounter the luminal side of the ER, are not glycosylated. As illustrated in Figure 7-35, the oligosaccharide is preformed in its entirety and is transferred to the target asparagine residue on the protein in a single enzymatic step almost as soon as that residue emerges on the luminal side of the ER membrane. This scheme ensures maximum access to the target asparagine (Asn) residues, which are those in the sequences Asn-X-Ser or Asn-X-Thr (where X is any amino acid). These two sequence combinations occur much less frequently in glycoproteins than in nonglycosylated cytoplasmic proteins. Evidently there has been selective pressure against these sequences during the evolution of glycoproteins, no doubt because glycosylation at many sites would interfere with protein folding.

If glycosylation occurred on the cytoplasmic surface, it might pose serious difficulties for the vectorial discharge mechanism, which would now have to accommodate bulky oligosaccharide chains. A system in which the oligosaccharide and the polypeptide are separately transported across the membrane by distinct mechanisms before being joined together on the luminal side would seem to simplify matters.



**Figure 7-31** The structure of the asparagine-linked oligosaccharide that is added to most proteins on the luminal side of the ER membrane. The sugars shown in color form the "core region" of this oligosaccharide. For many glycoproteins, only the core sugars survive the extensive oligosaccharide trimming process in the Golgi apparatus (see Figure 7-50).

### The Oligosaccharide Is Donated to the Polypeptide by an Activated Lipid and Then Almost Immediately Modified<sup>23</sup>

The sequence of events shown in Figure 7-35 requires that the oligosaccharide to be transferred to an asparagine residue must be present on the luminal side of the ER in an "activated" form. Activation is achieved by linking the oligosaccharide to a donor molecule via a high-energy bond. A major advance came in the early 1970s with the discovery that the activated donor is a special lipid molecule, **dolichol**, to which the oligosaccharide is linked via a pyrophosphate bridge.

The oligosaccharide is built up sugar by sugar on this membrane-bound lipid molecule. Sugars are first activated in the cytosol by the formation of *nucleotide-sugar intermediates*, which then donate their sugar (directly or indirectly) to the lipid in an orderly sequence (Figure 7-36). Dolichol is very